



NHP
RESEARCH
ALLIANCE



bapp.hyris.net/#/bkits

bKIT *Centella asiatica*

PN bKTB-CAS.01

Real-Time PCR assay

Hyris Ltd

Hyris Headquarters

Lower Ground Floor, One George Yard,
EC3V 9DF, London UK
Phone: +44.2036082968
Mail: office@hyris.net

Hyris Research Center

Corso Garibaldi, 60
20121 Milano, Italy
Phone: +39.02.82951302
Mail: administrator@hyris.net

Hyris Asia Pac

38 Ang Mo Kio Industrial Park 2 #02-07A
569511 Singapore
Phone: +65.8160.7207
Mail: office@hyris.net

www.hyris.net

Hyris Ltd
Hyris Headquarters

Lower Ground Floor, One George Yard,
 EC3V 9DF, London UK
 Phone: +44.2036082968
 Mail: office@hyris.net

Hyris Research Center

Corso Garibaldi, 60
 20121 Milano, Italy
 Phone: +39.02.82951302
 Mail: administrator@hyris.net

Hyris Asia Pac

38 Ang Mo Kio Industrial Park 2 #02-07A
 569511 Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications.

The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TagMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

Introduction

Centella asiatica, commonly known as gotu kola, is a plant species belonging to Apiaceae family. It is widely diffused in Asia where it has been used since ancient time in traditional medicine for treatments of various ailments. Modern analyses confirmed the presence of bioactive compounds¹, increasing the interest for its use as therapeutic agent for various skin conditions and for relieving anxiety and improving cognition².

(¹) Inamdar, E. K., Yeole, R. D., Ghogare, A. B. & Souza, N. J. Determination of biologically active constituents in *Centella asiatica*. *J Chromatogr A* 742, 124–130 (1996).

(²) Gohil KJ, Patel JA, Gajjar AK. Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. *Indian J Pharm Sci.* 2010 Sep;72(5):546-56. doi:10.4103/0250-474X.78519. PubMed PMID: 21694984; PubMed Central PMCID: PMC3116297.

Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Centella asiatica*. The product is intended for research purpose only.

NHPRA validation

The validation was performed on the bCUBE platform following the methods and the guidelines accepted and published on [J AOAC Int. 2019 Nov 1;102\(6\):1767-1773.](#)

In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following species were tested: *Bacopa monnieri*, *Merremia emarginata*, *Actaea racemosa*, *Coffea arabica*, *Curcuma longa* and *Vigna radiata*.

bKIT *Centella asiatica* packaging
Part number: bKTB-CAS.01-50

qPCR Master Mix (1 tube, blue cap)	50 tests
Positive Control (1 tube, green cap)	14 tests
Negative Control (1 tube, red cap)	14 tests

Part number: bKTB-CAS.01-100

qPCR Master Mix (2 tubes, blue cap)	2 x 50 tests
Positive Control (1 tube, green cap)	28 tests
Negative Control (1 tube, red cap)	28 tests

Storage

-20°C. Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the bKIT is correctly stored, at constant-temperature freezer, its performance is guaranteed until the shelf life indicated on the tubes.

Additional material/reagents required

- DNA extraction tools and reagents.
- Nuclease-free water.
- Gloves.
- Pipettes.
- bCUBE® instrument or other Real-Time PCR instrument (*) with filters calibrated for FAM.
- bCUBE® sample loading cartridge or, if using other Real-Time PCR instrument, samples loading support according to the instrument specifications.

(*) This assay was especially developed to be used in association with the bCUBE® instrument, available from Hyris Ltd, but can be used also with any other compatible thermal cyclers.

Hyris Ltd
Hyris Headquarters

Lower Ground Floor, One George Yard,
 EC3V 9DF, London UK
 Phone: +44.2036082968
 Mail: office@hyris.net

Hyris Research Center

Corso Garibaldi, 60
 20121 Milano, Italy
 Phone: +39.02.82951302
 Mail: administrator@hyris.net

Hyris Asia Pac

38 Ang Mo Kio Industrial Park 2 #02-07A
 569511 Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TagMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

DNA extraction

The assay has been validated on DNA samples obtained through the application of one or more protocols that are available for download on the bAPP.

The procedure for the download is the following:

- Login on the bAPP.
- Go on bKITs section and select **bKITB-CAS.01**.
- Click the button "Download DNA extraction method".

Reaction set-up

- Thaw all the bKIT components by placing the tubes on ice.
- Gently mix the tubes content by swirling the tubes.
- Spin the tubes to let the content down.
- In new tubes, one for each sample, including the **Negative Control** and the **Positive Control** of the bKIT, prepare the Reaction Mix as shown in the table below:

Components	Volume
DNA sample (diluted 1 in 10 after DNA extraction) or Positive Control or Negative Control	5 µL
qPCR Mastermix	15 µL
Total Volume	20 µL

Cartridge set-up

The procedure described is for the bCUBE® cartridge, but, if using a different Real-Time PCR instrument, the same procedure can be adopted for other loading sample supports with minor modifications.

1. Samples set-up

Samples of the following types must be prepared to be loaded on the cartridge:

- Positive Control** for *Centella asiatica*.
- Negative Control** for *Centella asiatica*.

Sample(s) to be tested.

2. Cartridge Loading

- Load the sample prepared as described in the previous section.
- Carefully seal the cartridge with adhesive film in order to avoid any contamination.
- Load the cartridge onto the bCUBE®, then start the run.

Analysis set-up

Set up the run method using the proper procedure, depending on the instrument you use.

1. On the bCUBE®

- Login on the bAPP/bPANEL.
 - If you are using the **bAPP**, set-up "New Analysis", flag bKIT, and select "**Botanicals**" → "**bKITB-CAS.01**"; alternatively, scan the QRCode on the front page of the present document, or on the bKIT packaging.
 - If you are using the **bPANEL**, set-up "New Analysis" and select "**Centella asiatica 1.x**" (x indicates the number of the last version available) from the "Global recipes" list.
- Specify the "Well types" for each of the loaded sample as follows (**Fig. 1**):
 - "PosCtrl" for the well loaded with *Centella asiatica*. **Positive Control**.
 - "NegCtrl" for the well loaded with *Centella asiatica*. **Negative Control**.
 - "Sample" for the wells loaded with samples under analysis.

Hyris Ltd

Hyris Headquarters

Lower Ground Floor, One George Yard,
EC3V 9DF, London UK
Phone: +44.2036082968
Mail: office@hyris.net

Hyris Research Center

Corso Garibaldi, 60
20121 Milano, Italy
Phone: +39.02.82951302
Mail: administrator@hyris.net

Hyris Asia Pac

38 Ang Mo Kio Industrial Park 2 #02-07A
569511 Singapore
Phone: +65.8160.7207
Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TagMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.



Fig 1. Cartridge set-up

An example of cartridge set-up on the bAPP for one replicate of a sample to be analyzed is shown.

2. On a compatible Real-Time PCR instrument

Please, contact us for the protocol set-up on the instrument.

Reading the results

1. On the bCUBE®

- a. The presence of the target *Centella asiatica* in the **Positive Control** or in the **sample** under analysis will generate an amplification curve (**Fig. 2**)

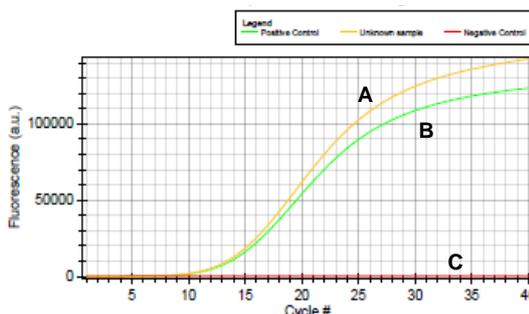


Fig.2. Amplification plot

In the plots, the amplification curve of a *Centella asiatica* containing sample (A), the Positive Control (B), and the Negative Control (C) are shown.

- b. At the end of analysis each well will be labelled depending on the “Well type” as described in the table below and samples classification will be shown on the pdf report of the analysis (**Fig. 3**).

Well type	Possible labels	Label meaning
Positive Control (PosCtrl)	OK	Specific amplification curve present
	KO	Specific amplification curve absent
Well type	Possible labels	Label meaning
Negative Control (NegCtrl)	OK	Specific amplification curve absent
	KO	Specific amplification curve present
Well type	Possible labels	Label meaning
Sample	Present	<i>Centella asiatica</i> is present in the sample
	Absent	<i>Centella asiatica</i> is absent from the sample
	Indeterminate	The test is not conclusive and should be repeated (**)

(**) If the “Indeterminate” classification persists, contact us at support@hyris.net.

Hyris Ltd
Hyris Headquarters

Lower Ground Floor, One George Yard,
 EC3V 9DF, London UK
 Phone: +44.2036082968
 Mail: office@hyris.net

Hyris Research Center

Corso Garibaldi, 60
 20121 Milano, Italy
 Phone: +39.02.82951302
 Mail: administrator@hyris.net

Hyris Asia Pac

38 Ang Mo Kio Industrial Park 2 #02-07A
 569511 Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

Results for target <i>Centella asiatica</i>	
Positive control (PosCtrl)	OK
Negative control (NegCtrl)	OK
Unknown sample (Sample)	Present

Fig.3. Analysis results table

An example of the results table, as reported in the pdf report of the analysis, is shown.

- On a compatible Real-Time PCR instrument
 Please, contact us for results interpretation.

Troubleshooting

- Results show no amplification, or anomalous amplification curves

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate/strips	Repeat the test using the appropriate materials and tools to seal correctly the plate/strips
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the supplier of the Real-Time PCR instrument
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, contact us at support@hyris.net .

- No amplification curve is observed for the Positive Control

Possible causes	Corrective actions
The Positive Control provided with the assay was not added into the reaction well	Repeat the test adding the Positive Control. If the problem persists, contact us at support@hyris.net .

- An amplification curve is observed for the Negative Control

Possible causes	Corrective actions
Contamination of the Negative Control or the qPCR Master Mix with target-positive DNA	Repeat the test by applying appropriate quality procedures to prevent contamination. Correctly seal the cartridge or plate/strips. If the problem persists, contact us at support@hyris.net .

Document revision Feb 06th, 2020